Citation:

Olmedilla-Alonso B, Granado-Lorencio F, Herrero-Barbudo C, Blanco-Navarro I, Blázquez-García S, Pérez-Sacristán B. Consumption of restructured meat products with added walnuts has a cholesterol-lowering effect in subjects at high cardiovascular risk: a randomised, crossover, placebo-controlled study. *J Am Coll Nutr*. 2008 Apr;27(2):342-8.

PubMed ID: 18689569

Study Design:

Randomized Crossover Trial

Class:

A - Click here for explanation of classification scheme.

Research Design and Implementation Rating:

NEUTRAL: See Research Design and Implementation Criteria Checklist below.

Research Purpose:

To assess the potential effect of regular consumption of walnut enriched restructured meat products on biomarkers of CHD [serum total, HDL and LDL cholesterol, triacylglycerols, homocysteine, vitamins B6 and B12, folic acid, α-tocopherol and platelet function test (obturation time)] in subjects at risk of CVD.

Inclusion Criteria:

Subjects had to have at least three of the cardiovascular risk factors indicated in the WHO/FAO report *Diet, Nutrition and the Prevention of Chronic Diseases*.

Inclusion criteria:

- Age (men: 45–65 y, women: 50–70 y, postmenopausal)
- Overweight (BMI >25 and <34.90 kg/m2
- Serum cholesterol >220 and <290 mg/d

And at least one of the following features:

- Smoking habit
- Blood pressure ~140/90 mm Hg

The habitual consumption of a mixed diet (no avoidance of any food groups) was required.

Informed consent was obtained from the study subjects.

Exclusion Criteria:

Exclusion criteria

Use of:

- Vitamin or mineral supplements
- Hormone replacement therapy
- Regular aspirin regimen
- Medications known to affect lipid absorption or metabolism

In addition:

- Any chronic disease (i.e. diabetes)

Individuals with blood pressure over 145/95 mm Hg or taking medication for hypertension were initially excluded, but, given the small number of subjects willing to participate, it was necessary to include patients being treated for this condition (n=4, taking enalaprilate, lacidipine or candesartan).

Description of Study Protocol:

Recruitment

Twenty-five volunteers (15 men, 10 women) were selected from among the 144 who responded to the advertisements of the intervention study. Although many subjects were conscious of displaying three or more risk factors for CVD (target group), the response of the potential volunteers was far from ideal, with an intent to participate of less than 30% of those contacted. Twenty-two volunteers were excluded for different reasons, including the habitual use of drugs to treat hypercholesterolemia, diabetes and other chronic diseases.

Design: Randomized crossover trial

In order to evaluate the potential functionality of the food/nutrients incorporated, two approaches were employed:

Protocol 1

The postprandial (single-dose) bioavailability of walnut components contained in the new product was assessed using a marker of exposure (i.e., γ-tocopherol).

Three of the volunteers participated in a crossover study with the walnut-enriched restructured meat product to evaluate the postprandial response of γ-tocopherol in triacylglycerol-rich fractions over a 7-hour period (γ-tocopherol is used as an exposure biomarker because nuts are a particularly rich source of this compound, which is not abundant in the Spanish diet).

Protocol 2

A crossover unblinded dietary intervention study (long-term bioavailability of α-tocopherol) to assess the effect on intermediate risk markers in a group considered at risk for CVD.

The dietary intervention protocol consisted of a crossover, multiple-dose study involving restructured meat products with or without walnuts (active vs control): 150 g steak (4 per week) and 80 g frankfurter (1 per week) over a 5-week study period (regular consumption), with a 1-month washout in between.

These meat products were delivered frozen, and were to be grilled with ca. 10 ml olive oil. The volunteers were asked to continue with their habitual diets and lifestyle, but substituting the restructured meat products being studied for their usual meat servings and avoiding the intake of walnuts apart from those incorporated into the meat. The volunteers completed a diet record throughout the study in order to allow researchers to check the compliance with the intervention and to confirm the substitution of the meat products in their ordinary meals, while maintaining a mixed diet throughout the study periods.

The equivalent walnut consumption would be 30 g of walnut per steak and 16 g of walnut per serving of frankfurters, which amounts to a consumption of 136 g of walnuts a week, for a mean consumption of 19.4 g a day. The weekly γ-tocopherol intake with these meat products was approximately 62.40 to 72.00 μmol.

Overnight fasting blood samples were collected from all the participants at baseline and on days 12, 21, 28 and 35, and from some of them approximately at the midpoint of the washout period (about day 47). During each visit to the

hospital, the blood samples and dietary records of each participant were collected, the blood pressure and body weight were recorded, and the subjects were given a new lot of meat products and a diet record.

Blinding used: Unblinded

Intervention

Dietary intervention consisted of regular consumption of the meat product, with or without walnuts, five times per week for five weeks with a 1-month washout in between.

Statistical Analysis

Power analysis:

On the basis of a mean value for the baseline total cholesterol of 230 mg/dl, a sample size of 26 subjects was shown to provide an 80% power to detect an expected decrease of 5% in total cholesterol (11.5 mg/dl) with the consumption of nuts, at an alpha level of 0.05.

Statistical test:

Results are expressed as mean ±SD. The normal distribution of the data was assessed using the Kolmogorov-Smirnov test.

There were no significant differences either between men and women in terms of the analyses evaluated at baseline, or in those analyses in each volunteer at the beginning of the two studies (Student's T test). Thus, the statistical analyses of the variables studied in men and women were performed jointly. In addition, the baseline value for each analysis was the mean of the two samples from each volunteer collected at the beginning of each of the two studies.

Since diet-induced lipoprotein changes stabilize in less than four weeks, and the researchers found no differences between the two final points in the intervention (days 28 and 35), the mean of these two final values were used to assess changes in the response variables - the concentrations of total cholesterol, LDL cholesterol, HDL cholesterol and γ-tocopherol-between the baseline and the end of the intervention periods.

Student's t test for paired data (two-tailed) was used to assess the significance of percentage changes across diets and compared to baseline.

All the statistical analyses were performed with the SPSS statistical package vs. 12 (SPSS Inc. IL, USA). A p value <0.05 was considered to indicate statistical significance.

Data Collection Summary:

Timing of Measurements

Overnight fasting blood samples were collected from all the participants at baseline and on days 12, 21, 28 and 35, and from some of them approximately at the midpoint of the washout period (about day 47). During each visit to the hospital, the blood samples and dietary records of each participant were collected, the blood pressure and body weight were recorded, and the subjects were given a new lot of meat products and a diet record.

Dependent Variables

Biomarkers related to CHD (biomarkers of function, risk indicators and related markers):

- Serum cholesterol (total, HDL cholesterol and LDL cholesterol)
- Triacylglycerols
- Homocysteine
- Vitamins B6, B12, folic acid, γ-tocopherol, γ-tocopherol/α-tocopherol

- HDL/LDL ratio
- Platelet function test (obturation time)

Independent Variables

The regular consumption of walnut-enriched meat products compared with that of the restructured meat products without added walnuts.

Restructured meat products with or without walnuts (active vs control): 150 g steak (4 per week) and 80 g frankfurter (1 per week) over a 5-week study period (regular consumption), with a 1-month washout in between.

The equivalent walnut consumption would be 30 g of walnut per steak and 16 g of walnut per serving of frankfurters, which amounts to a consumption of 136 g of walnuts a week, for a mean consumption of 19.4 g a day. The weekly γ-tocopherol intake with these meat products was approximately 62.40 to 72.00 μmol.

Control Variables

Description of Actual Data Sample:

Initial N:

25 (15 males, 10 females)

Tables list different numbers of subjects for several of the biomarker tests without explanation for the variance from the 25 subjects listed in the Subjects section. Number of subjects: folic acid (n=14), vitamin B6 (n=30), vitamin B12 (n=14), platelet function test (n=14). Perhaps there were problems with the biological samples but there was no discussion of this.

Attrition (final N): as above

Age: 54.4 ± 8.1

Ethnicity:

Subjects were recruited in Madrid, Spain. However, there is no mention of ethnicity in the research article.

Other relevant demographics:

17 non-smokers and 8 smokers. The results combined the data for non-smokers and smokers.

Subjects served as their own controls so there were no demographic differences between groups.

Anthropometrics

Subjects served as their own controls.

Smoking and gender differences were not compared or reported.

Location: Madrid, Spain.

Summary of Results:

Key Findings

- The regular consumption of walnut-enriched meat products compared with that of the restructured meat products without added walnuts causes a significant decrease in total cholesterol of 6.8 mg/dl (CI95%: -12.8,-0.85).
- Compared to baseline (mixed diet), meat products with walnuts significantly decreased:
  - Total cholesterol (-10.7 mg/dl, CI95%: -17.1, -4.2)
  - LDL cholesterol (-7.6 mg/dl, CI95%: -2.2, -13.0)
  - Body weight (-0.5 kg, CI95%: -0.1, -0.9)
- Compared to baseline (mixed diet), meat products with walnuts significantly increased γ-tocopherol (8.9 mg/dl, CI95%: 1.0, 16.8).

Serum Concentrations (Mean ± SD) of Biomarkers Assessed in the Intervention Study

	Baseline	Final concentrations (walnuts) (a)	Final concentrations (without walnuts) (control) (b)	Difference between final concentrations (a) and (b)
Total cholesterol (mg/dl) (c) Difference from baseline (CI95%), p	235.9 ± 41.1	225.3 ± 38.6 -10.7 (-17.1, -4.2) p = 0.002	232.1 ± 44.2 -3.8 (-11.5, 3.8) p = 0.309	-6.8 (-12.8,-0.85) p = 0.027
HDL cholesterol (mg/dl) (c) Difference from baseline (CI95%), p	53.5 ± 14.3	51.8 ± 12.8 -1.7 (-3.9, 0.6) p = 0.139	53.5 ± 14.3 -0.4 (-2.0, 1.1) p = 0.581	-1.3 (-3.4, 0.9) p = 0.334
LDL cholesterol (mg/dl) (c) Difference from baseline (CI95%), p	151.9 ± 32.5	144.2 ± 29.3 -7.6 (-2.2,-13.0) p = 0.007	148.8 ± 34.1 -3.1 (-11.2, 5.1) p = 0.448	-4.6 (-13.6, 4.4) p = 0.303
Triacylglycerols (mg/dl) (c) Difference from baseline (CI95%), p	149.2 ± 88.0	142.9 ± 64.3 -6.32 (-28.0, 15.3) p = 0.552	158.8 ± 88.7 7.4 (-15.7, 30.5) p = 0.515	-15.9 (-41.4, 9.5) p = 209
γ-Tocopherol (µg/dl) (c) Difference from baseline (CI95%), p	43.7 ± 18.1	52.6 ± 19.4 8.9 (1.0, 16.8) p = 0.029	47.2 ± 20.7 3.5 (-3.7, 10.8) p = 0.324	5.4 (-4.1, 14.8) p = 0.255
α-tocopherol (µg/dl) Difference from baseline (CI95%), p	1334.8 ± 319.9	1296.8 ± 270.4 -35.9 (-90.6, 18.7) p = 0.187	1347.0 ± 347.9 10.2 (-67.6, 88.0) p = 0.789	-50,2 (-144.7,-44.3) p = 0.284
Body weight (kg) Difference from baseline (CI95%), p	82.1 ± 12.9	81.6 ± 12.9 -0.50 (-0.96,-0.05) p = 0.032	81.8 ± 12.8 -0.31 (-4.4, 3.7) p = 0.878	-0.20 (-4.3, 3.9) p = 0.920
Blood pressure (mm Hg) Systolic Difference from baseline (CI95%), p	143.5 ± 16.9	138.6 ± 22.8 -2.3 (-12.2, 3.7) p = 0.277	133.3 ± 16.8 -10.8 (-16.4, 5.2) p = 0.001	4.5 (-2.9, 11.9) p = 0.216
Diastolic Difference from baseline (CI95%), p	86.3 ± 10.4	85.0 ± 12.1 -2.4 (-1.7, 0.9) p = 0.099	87.3 ± 17.1 -0.4 (-7.4, 6.7) p = 0.920	-0.21 (-5.6, 5.2) p = 0.936

(c) Baseline and final concentrations of total cholesterol, LDL cholesterol, HDL cholesterol and γ-tocopherol correspond to the mean of two determinations for each volunteer; baseline data correspond to the mean of the data for each subject at the start of the two assays and final data correspond to the mean of analyses on days 28 and 35.

Other Findings

- The researchers carried out a single-dose, crossover bioavailability study to determine the bioavailability of γ-tocopherol. γ-tocopherol is used as an exposure biomarker because nuts are a particularly rich source of this compound which is not abundant in the Spanish diet.
- The consumption of a restructured meat product led to an increase in γ-tocopherol in the TRL fraction during the postprandial period (maximum increment: 16.3 µg/dl) only when walnuts were added. The increase reached the maximal value 6 hours after intake (time evaluated: 6 hours). This provides *in vivo* evidence of the efficacy of walnut-enriched restructured meat products as a vehicle for bioactive substances (i.e., γ-tocopherol).

Author Conclusion:

The restructured meat products with added walnuts supplied in this study can be considered functional foods for subjects at high risk for CVD, as their regular consumption induces a small but significant reduction in total cholesterol of 4.5% with respect to baseline values (mixed diet) and 3% with respect to the restructured meat without walnuts.

The authors claim that the present study is to their knowledge the first attempt to validate, in humans, a potential meat-based walnut-enriched functional product designed to diminish CHD risk, using biomarkers for exposure and function.

In contrast to previous human trials with walnuts, this study involves a number of portions of meat per week consistent with the current consumption patterns in most western countries, while the amount of walnuts provided is approximately 70% of a daily intake that could contribute to diminish risk of CHD, according to the FDA.

The present intervention study took into account two recommendations for the design of trials to examine effects of fatty foods on cardiovascular risk factors: a duration of at least 3 to 4 weeks and a break between interventions to ensure a stable endpoint measurement.

In summary, the authors conclude that since the regular consumption of restructured meat products containing walnuts causes a reduction of the intermediate clinical markers of CHD (total and LDL cholesterol with respect to the baseline mixed diet and total cholesterol with respect to the restructured meat products without walnuts), they can be considered promising products that meet the criteria for functional foods for subjects at high risk for CVD. However, before any claims about these meat products can be made with respect to cardiovascular risk, it will be necessary to reproduce the same type of study with other subjects of the same characteristics and, in any case, comply with legislation in each country.

Reviewer Comments:

Researchers had difficulties enrolling subjects. Smoking was not controlled for, and there was uneven gender distribution.

In addition, although the effects were significant, they were not particularly large.

Finally, there was no discussion of palatability of the restructured product with or without walnuts. (However, they did refer to previous studies of these products which may have discussed this issue.)

Research Design and Implementation Criteria Checklist: Primary Research

Relevance Questions		
1.	Would implementing the studied intervention or procedure (if found successful) result in improved outcomes for the patients/clients/population group? (Not Applicable for some epidemiological studies)	Yes
2.	Did the authors study an outcome (dependent variable) or topic that the patients/clients/population group would care about?	Yes

3.	Is the focus of the intervention or procedure (independent variable) or topic of study a common issue of concern to nutrition or dietetics practice?	Yes
4.	Is the intervention or procedure feasible? (NA for some epidemiological studies)	Yes

Validity Questions

1.	**Was the research question clearly stated?**	Yes
1.1.	Was (were) the specific intervention(s) or procedure(s) [independent variable(s)] identified?	Yes
1.2.	Was (were) the outcome(s) [dependent variable(s)] clearly indicated?	Yes
1.3.	Were the target population and setting specified?	Yes
2.	**Was the selection of study subjects/patients free from bias?**	???
2.1.	Were inclusion/exclusion criteria specified (e.g., risk, point in disease progression, diagnostic or prognosis criteria), and with sufficient detail and without omitting criteria critical to the study?	Yes
2.2.	Were criteria applied equally to all study groups?	No
2.3.	Were health, demographics, and other characteristics of subjects described?	Yes
2.4.	Were the subjects/patients a representative sample of the relevant population?	No
3.	**Were study groups comparable?**	Yes
3.1.	Was the method of assigning subjects/patients to groups described and unbiased? (Method of randomization identified if RCT)	Yes
3.2.	Were distribution of disease status, prognostic factors, and other factors (e.g., demographics) similar across study groups at baseline?	Yes
3.3.	Were concurrent controls used? (Concurrent preferred over historical controls.)	Yes
3.4.	If cohort study or cross-sectional study, were groups comparable on important confounding factors and/or were preexisting differences accounted for by using appropriate adjustments in statistical analysis?	N/A
3.5.	If case control or cross-sectional study, were potential confounding factors comparable for cases and controls? (If case series or trial with subjects serving as own control, this criterion is not applicable. Criterion may not be applicable in some cross-sectional studies.)	N/A

3.6.	If diagnostic test, was there an independent blind comparison with an appropriate reference standard (e.g., "gold standard")?	N/A
4.	**Was method of handling withdrawals described?**	Yes
4.1.	Were follow-up methods described and the same for all groups?	Yes
4.2.	Was the number, characteristics of withdrawals (i.e., dropouts, lost to follow up, attrition rate) and/or response rate (cross-sectional studies) described for each group? (Follow up goal for a strong study is 80%.)	Yes
4.3.	Were all enrolled subjects/patients (in the original sample) accounted for?	Yes
4.4.	Were reasons for withdrawals similar across groups?	N/A
4.5.	If diagnostic test, was decision to perform reference test not dependent on results of test under study?	N/A
5.	**Was blinding used to prevent introduction of bias?**	Yes
5.1.	In intervention study, were subjects, clinicians/practitioners, and investigators blinded to treatment group, as appropriate?	No
5.2.	Were data collectors blinded for outcomes assessment? (If outcome is measured using an objective test, such as a lab value, this criterion is assumed to be met.)	Yes
5.3.	In cohort study or cross-sectional study, were measurements of outcomes and risk factors blinded?	N/A
5.4.	In case control study, was case definition explicit and case ascertainment not influenced by exposure status?	N/A
5.5.	In diagnostic study, were test results blinded to patient history and other test results?	N/A
6.	**Were intervention/therapeutic regimens/exposure factor or procedure and any comparison(s) described in detail? Were interveningfactors described?**	Yes
6.1.	In RCT or other intervention trial, were protocols described for all regimens studied?	Yes
6.2.	In observational study, were interventions, study settings, and clinicians/provider described?	N/A
6.3.	Was the intensity and duration of the intervention or exposure factor sufficient to produce a meaningful effect?	Yes
6.4.	Was the amount of exposure and, if relevant, subject/patient compliance measured?	Yes
6.5.	Were co-interventions (e.g., ancillary treatments, other therapies) described?	N/A
6.6.	Were extra or unplanned treatments described?	N/A

6.7.	Was the information for 6.4, 6.5, and 6.6 assessed the same way for all groups?	Yes
6.8.	In diagnostic study, were details of test administration and replication sufficient?	N/A
7.	**Were outcomes clearly defined and the measurements valid and reliable?**	Yes
7.1.	Were primary and secondary endpoints described and relevant to the question?	Yes
7.2.	Were nutrition measures appropriate to question and outcomes of concern?	Yes
7.3.	Was the period of follow-up long enough for important outcome(s) to occur?	Yes
7.4.	Were the observations and measurements based on standard, valid, and reliable data collection instruments/tests/procedures?	Yes
7.5.	Was the measurement of effect at an appropriate level of precision?	Yes
7.6.	Were other factors accounted for (measured) that could affect outcomes?	???
7.7.	Were the measurements conducted consistently across groups?	Yes
8.	**Was the statistical analysis appropriate for the study design and type of outcome indicators?**	Yes
8.1.	Were statistical analyses adequately described and the results reported appropriately?	Yes
8.2.	Were correct statistical tests used and assumptions of test not violated?	Yes
8.3.	Were statistics reported with levels of significance and/or confidence intervals?	Yes
8.4.	Was "intent to treat" analysis of outcomes done (and as appropriate, was there an analysis of outcomes for those maximally exposed or a dose-response analysis)?	N/A
8.5.	Were adequate adjustments made for effects of confounding factors that might have affected the outcomes (e.g., multivariate analyses)?	???
8.6.	Was clinical significance as well as statistical significance reported?	Yes
8.7.	If negative findings, was a power calculation reported to address type 2 error?	N/A
9.	**Are conclusions supported by results with biases and limitations taken into consideration?**	Yes
9.1.	Is there a discussion of findings?	Yes
9.2.	Are biases and study limitations identified and discussed?	Yes
10.	**Is bias due to study's funding or sponsorship unlikely?**	Yes

10.1.	Were sources of funding and investigators' affiliations described?	Yes
10.2.	Was the study free from apparent conflict of interest?	Yes